TECHNICAL COMMENT

A Field Validation of Plasma Metabolite Profiling to Assess Refueling Performance of Migratory Birds

Christopher G. Guglielmo*
David J. Cerasale
Charles Eldermire
Division of Biological Sciences, University of Montana, Missoula, Montana 59812

Accepted 3/1/04

ABSTRACT

Plasma metabolite profiling offers a potential means to assess stopover refueling performance of migratory birds from a single capture. However, this method has not previously been validated where site quality has been determined independently using analysis of capture data. We captured and blood sampled six passerine bird species refueling at known high-quality (BASE) and low-quality (TIP) sites at Long Point, Ontario, Canada. Plasma triglyceride, an indicator of fat deposition, was higher at the BASE in three early-season species: the hermit thrush, the American robin, and the white-throated sparrow. Plasma B-OH-butyrate, an indicator of fasting and lipid utilization, was lower at the BASE in the same three species. Plasma glycerol was lower at the BASE in American robins, and plasma phospholipid did not differ between sites. No metabolite suggested better conditions at the TIP in any species. Regression of size-corrected mass on time of day also indicated better refueling performance at the BASE in some species, but metabolite profiling was generally more sensitive to site differences. The relationship between plasma glycerol and triglyceride was U-shaped, indicating high glycerol production during both lipolysis (as was previously known) and rapid fat deposition. Our results confirm the validity of metabolite profiling to assess stopover habitat quality and individual performance in refueling migrants.

* Corresponding author; present address: Department of Biology, University of Western Ontario, London, Ontario N6A 5B7, Canada; e-mail: chris.guglielmo@mso.umt.edu.

Physiological and Biochemical Zoology 78(1):116–125. 2005. 1522-2152/2005/7801-3135$15.00

Introduction

The migratory journeys of birds generally consist of alternating bouts of intense endurance exercise and hyperphagic refueling at stopover sites. Refueling rate is thought to be a major determinant of stopover duration, departure fuel load, and overall migration speed, and it ultimately affects survival and reproduction of migratory birds (Alerstam and Lindström 1990; Alerstam and Hedenström 1998). Fuel deposition rate is not easily determined under field conditions, and typically some analysis of capture data is used. In the special case of synchronous migrants, mass or body composition changes can be followed through time (Lindström and Piersma 1993). More commonly, the daily rate of mass change is determined by weighing birds captured multiple times (Biebach et al. 1986; Moore and Kerlinger 1987) or from the slope of a regression of absolute body mass on the time of day when the bird was captured (Yong et al. 1998; Dunn 2000, 2001, 2002). Problems with the recapture method include a large required capture effort (only a small percentage is typically recaptured) and potential biases associated with (1) capture stress affecting mass change between captures and (2) increased probability of recapturing low-quality individuals that may remain at a site longer. Regression methods may also require a large capture effort to obtain reliable estimates, and they are not suitable for species whose feeding behavior depends on other factors in addition to light conditions. For example, tides strongly influence feeding behavior of shorebirds (Guglielmo et al. 2002*a*).

Plasma metabolite profiling offers a physiological means to assess the instantaneous refueling rate of individual birds from a single capture based on the principle that metabolic state (feeding, fasting) is reflected in the levels of circulating metabolites (Jenni-Eiermann and Jenni 1994; Williams et al. 1999). Metabolites involved in fat deposition and mobilization appear to be particularly informative in migrants. For example, during feeding and fat deposition, plasma triglyceride (TRIG) concentration rises as lipids are absorbed in the gut or synthesized de novo in the liver. Plasma TRIG has also been demonstrated to rise during endurance flight in some birds, but this is not a major factor for refueling studies provided birds are not captured just after landing (Jenni-Eiermann and Jenni 1991, 1992). During fasting and mass loss, glycerol (GLYC) and B-OH-butyrate (BUTY) levels increase due to high rates of lipolysis and ketone formation, respectively. Studies with captive birds

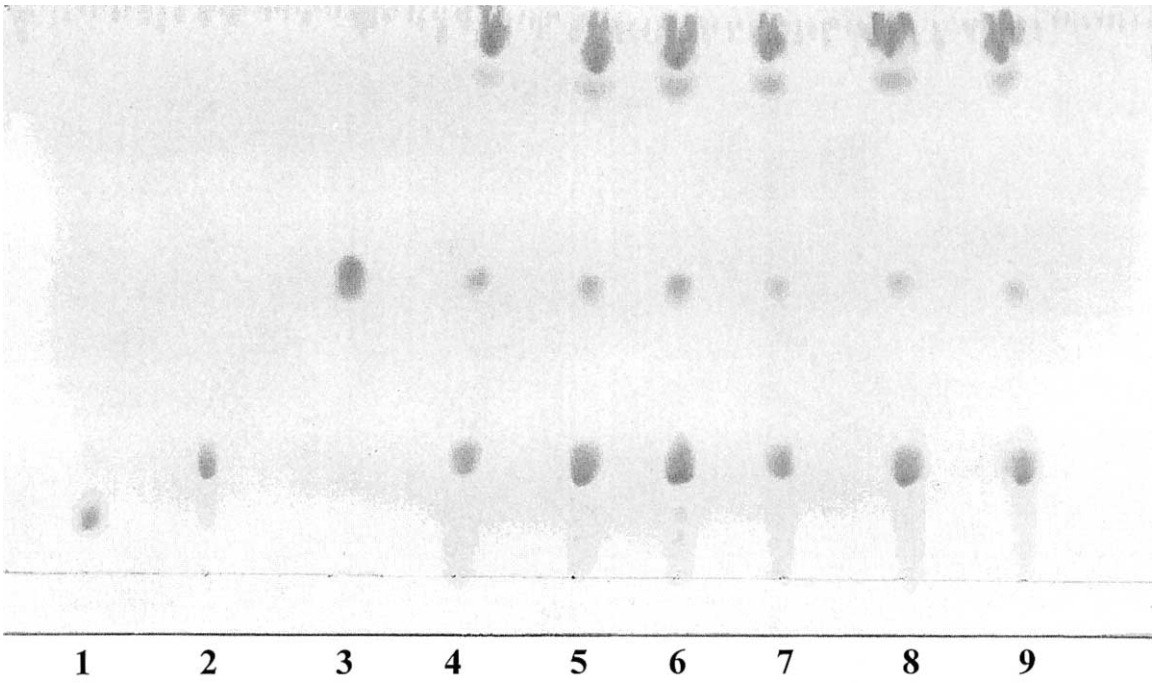


Figure 1. Plasma phospholipids resolved by thin-layer chromatography on silica gel with chloroform : methanol : water 70 : 25 : 3. Standards (12 μg) are sphingomyelin (*1*), phosphatidyl choline (*2*), and phosphatidyl ethanolamine (*3*). Bird samples are American robin (*4*), gray catbird (*5*), hermit thrush (*6*), Swainson's thrush (*7*), white-throated sparrow (*8*), and magnolia warbler (*9*). Unidentified lipids near the top of the plate contain neutral lipids and nonesterified fatty acids.

demonstrate that these three metabolites can be used to predict mass change over periods of hours to a few days (Jenni-Eiermann and Jenni 1994; Williams et al. 1999; Jenni and Schwilch 2001). Field studies show that plasma TRIG levels are elevated during migration periods (Jenni and Jenni-Eiermann 1996; Guglielmo et al. 2002*a*, 2002*b*) and that metabolite profiles can differ among stopover sites, suggesting corresponding differences in refueling rate (Schaub and Jenni 2001; Guglielmo et al. 2002*a*). Evidence from free-living birds also indicates that elevated plasma phospholipid (PL) may reflect fat deposition, and in combination with TRIG it may provide information about diet lipid composition (Guglielmo et al. 2002*b*). Invertebrates store surplus energy as TRIG, whereas whole-body PL is a function of membrane content and thus body size (Hentschel 1998). High-quality invertebrates should have a higher ratio of TRIG to PL than those with low energy stores. Bird plasma TRIG : PL may in turn be related to the nutritional state of prey. In western sandpipers (*Calidris mauri*), the plasma neutral lipid (mainly TRIG) to PL ratio increased between spring and fall migration and was hypothesized to reflect an over-summer increase in triglyceride stores of benthic invertebrates (Guglielmo et al. 2002*b*).

Many studies have focused on the causes of variation in the metabolites themselves (e.g., mass, age, sex, feather molt, migratory state, fasting, feeding, and flying). Recently, metabolite profiles have begun to be applied in an ecological context as indices of bird refueling performance at stopover sites (Schaub and Jenni 2001; Ydenberg et al. 2002). While underlying support from captive studies is robust, it has never been demonstrated under field conditions that plasma metabolite profiles differ in the expected manner between stopover sites of known quality. Furthermore, relationships between plasma metabolites and mass change have only been studied in species that mainly consume invertebrates. The goal of this study was to test the validity of plasma metabolite profiling at refueling sites shown previously to be of high and low quality using several bird species representing a diversity of migration and feeding strategies. In addition, we examined the potential utility of plasma PL as an indicator of mass gain.

Material and Methods

We conducted field studies at the Long Point Bird Observatory (LPBO), Ontario, Canada from April 6 to June 4, 2002. Long Point is a 35-km peninsula extending eastward from the north shore of Lake Erie, and it is an attractive stopover for migratory birds. Previous regression analyses of 17 yr of capture data by Dunn (2000, 2001) demonstrated that spring refueling rates are higher in a wide variety of bird species at a site near the base (BASE) of Long Point than at the tip (TIP). The BASE

Table 1: Sample sizes, body masses, and median dates of capture of hermit thrushes (HETH), white-throated sparrows (WTSP), American robins (AMRO), gray catbirds (GRCA), magnolia warblers (MAWA), and Swainson's thrushes (SWTH) sampled at the base and tip of the Long Point peninsula, Ontario, Canada

	BASE			TIP		
Species	*N*	Mass (g)	Median Date	*N*	Mass (g)	Median Date
HETH[a]	46	29.8 ± .3	April 16	28	28.3 ± .5	May 2
WTSP	47	27.9 ± .3	April 23	48	27.2 ± .3	April 30
AMRO	8	78.3 ± 2.4	April 12	10	77.6 ± 1.9	May 6
GRCA	17	35.7 ± .6	May 23	13	35.0 ± .5	May 7
MAWA	30	8.4 ± .2	May 23	10	8.2 ± .3	May 24
SWTH[a]	13	32.3 ± .8	May 26	15	34.9 ± .8	May 26

Note. Species are ordered from top to bottom according to overall median date of capture.
[a] Body mass differed between BASE and TIP (HETH, $P = 0.002$; SWTH, $P = 0.03$).

site is located in a residential area and is a mixture of woodland and low shrubs. The TIP is characterized by sand beach, dunes, and marsh with a patchy cover of shrubs and trees. The TIP is more subject to cold spring temperatures and high winds, which delay the phenology of plants and invertebrates (Dunn 2000).

We chose six species to sample in coordination with normal LPBO capture and marking activities; nets opened 30 min before sunrise and usually closed 6.5 h later. Because of the distance between sites (30 km), birds marked at one site are rarely ever caught at the other, especially on the same day. The American robin (*Turdus migratorius*) and gray catbird (*Dumetella carolinensis*) usually migrate early in spring, eat mainly fruit and invertebrates, and are known to breed in the study area as well as farther north. The hermit thrush (*Catharus guttatus*) and Swainson's thrush (*Catharus ustulatus*) are very long-distance migrants that feed primarily on understory and terrestrial invertebrates and fruits. The hermit thrush migrates earlier in spring than the Swainson's thrush, and both species typically breed north of the study area. The magnolia warbler (*Dendroica magnolia*) is a small long-distance migrant insectivore that breeds primarily north of the study area. The white-throated sparrow (*Zonotrichia ablicollis*) is a granivore that will also consume insects during migration. It generally migrates early in spring and breeds north of Long Point.

We continuously monitored mist nets with a timer in order to determine as accurately as possible the delay between capture and blood sampling. Most birds were seen to enter a net, and the remaining birds were detected only a few minutes after capture. Birds were removed from mist nets, placed in a light cotton bag, and carried to a bleeding station. Approximately 10% of blood volume was collected by brachial veinipuncture with a 26-gauge needle. For most birds, blood was collected into a one-step collection/centrifuge tube (microvette CB 300, Li heparin, Sarstedt, Newton, NC). Blood was collected from magnolia warblers into heparizined microhematocrit tubes as described previously (Guglielmo et al. 2002*a*). Blood was centrifuged at 2,000 *g* for 10 min, and the plasma was stored in screw top 0.6 mL cryogenic tubes in a liquid N_2 dry shipper. Birds were weighed (± 0.1 g) and measured (wing $\pm$ 0.5 mm and tarsus $\pm$ 0.1 mm), and then aged, sexed, and banded by LPBO staff according to Pyle et al. (1987). Plasma was stored at -80°C for 4 mo until analysis. All sample collections were permitted by the Canadian Wildlife Service, and animal handling procedures were approved by the University of Montana Institutional Animal Care and Use Committee.

Metabolites were assayed on a microplate spectrophotometer (Biotec Powerwave X 340) in 400 μL flat-bottom microplates (Nunc, Roskilde, Denmark). GLYC and TRIG were measured sequentially by endpoint assay (SIGMA, Trinder reagent A and B) as described previously (Guglielmo et al. 2002*a*). BUTY was measured by kinetic endpoint assay (kit E0907979, R-Biopharm, Marshall, MI) at room temperature (22°–25°C) as follows: 5 μL standard or sample, 200 μL working solution, measure ΔA/min 492 nm for 2 min to check for high background, rapidly add 4 μL B-OH-butyrate dehydrogenase suspension to all wells with a positive displacement repeater pipette (Eppendorf), measure ΔA 492 nm 0–40 min, subtract three times ΔA 30–40 min (background) from ΔA 0–30, calculate against linear standard curve 0.15–3.17 mmol/L BUTY. Plasma PL were measured by endpoint assay using a colorimetric kit (phospholipids B, WAKO Diagnostics, Richmond, VA) that measures free choline following phospholipase D degradation. Thus the kit can detect phosphatidyl choline (PC), lysophosphatidyl choline (LPC), and sphingomyelin (SM), but not phosphatidyl ethanolamine (PE). Assay conditions were 2.5 μL standard (0–7.74 mmol/L choline) or sample (diluted threefold with 0.9% NaCl), 300 μL reagent (prewarmed to 37°C), incubate 10 min at 37°C, and measure absorbance at 505 nm and 700 nm (secondary).

Thin-layer chromatography (TLC) was used to measure the

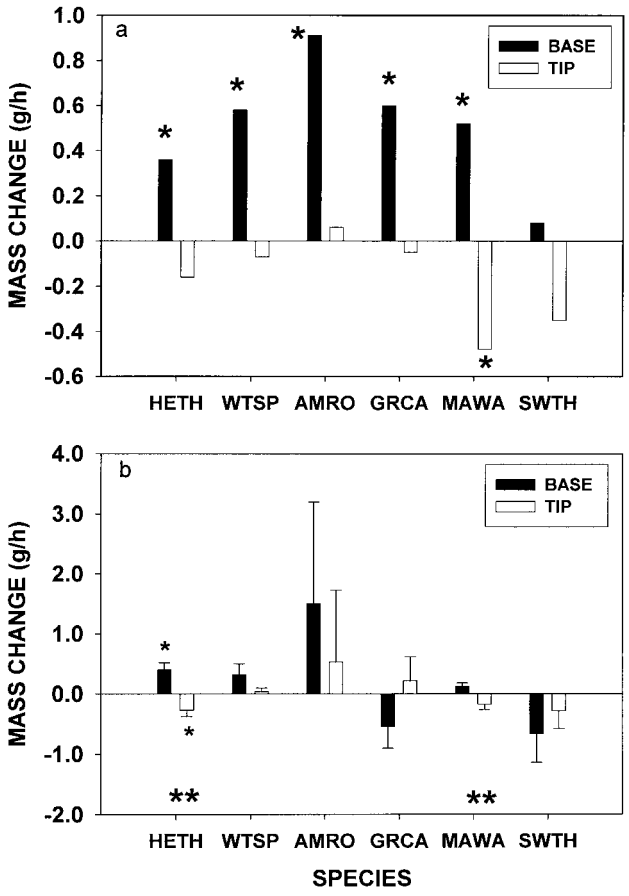


Figure 2. Rates of change of body mass estimated by regression of body mass against time of day, controlling for body structural size for birds refueling at the BASE and TIP study areas of Long Point. Species abbreviations and sample sizes as in Table 1. Species are ordered from left to right according to overall median date of capture. *a*, rates reported in Table 1 of Dunn (2001); an asterisk indicates significantly different from zero mass change. *b*, rates (±SE) estimated from measurements of birds captured and blood sampled for the present study in 2002; one asterisk indicates significantly different from zero; two asterisks indicate a significant difference between BASE and TIP.

relative amounts of the major PL classes to determine if the kit provides a reliable index of total plasma PL of birds. Total plasma lipids were extracted by adding 80–100 μL plasma to 4 mL chloroform : methanol 2 : 1 in a 12 mL glass culture tube. Tubes were centrifuged 10 min at 2,060 *g*, filtered (Whatman #1), pellet re-extracted, and then filtered with 2 mL of solvent. Then 1.5 mL of 0.25% KCl was added to the filtrate, and after vigorous mixing and incubation for 5 min in a 70°C water bath, the aqueous phase was removed by suction. The extract was dried under N_2 at 70°C and dissolved in 100 μL chloroform for loading (6 μL) onto 200 μm silica gel plates (SIGMA). The developing solvent was chloroform : methanol : water 70 : 25 : 3, and spots were detected with iodine. Spots were identified by comparison with PC, PE and SM standards (12 μg each; SIGMA). The purity of spots was confirmed by two dimensional TLC of selected samples using chloroform : methanol : acetic acid : water 80 : 9 : 12 : 2 as a secondary solvent, but we found one-dimensional TLC to be sufficient. Plates were digitally scanned (HP 4470c) and analyzed using National Institutes of Health Image 1.63. Our analysis showed that the composition of plasma PL was similar across species with PE comprising 26% ± 1.6%, PC 60.8% ± 7.7%, SM 7.7% ± 2.6%, and a very polar PL (likely LPC) 5.4% ± 1.9% of total plasma PL (Fig. 1). Total choline containing PL (PC + SM + LPC) was not significantly correlated to PE content ($r = 0.28$, $P = 0.37$). However, PC + SM + LPC was strongly positively correlated with total PL ($r = 0.84$, $P = 0.0006$), leading us to conclude that the PL assay kit will detect most bird plasma PL.

Differences in absolute body mass between the BASE and TIP were tested by ANCOVA with mass as a dependent variable, site an independent variable, and body size as a covariate. Age and sex were not considered in the analysis because they could not be determined definitively and sample sizes were low. The first principal component score (PC1) of a PCA of tarsus and wing length was used as a measure of body size (Rising and Somers 1989). We estimated hourly rate of mass change from our capture data by multiple regression of body mass on time since sunrise and body size (Dunn 2001). Differences in rate of mass change between the BASE and TIP were detected by entering site into the model and testing for an interaction between site and time since sunrise. TRIG, GLYC, and BUTY data were transformed to $\log_{10}+1$ to satisfy normality assumptions. Site differences in plasma metabolite concentrations were tested by multiple regression with backward selection. Site was coded as a dummy variable (0, 1) and variables (site, mass, bleed time, date, and time since sunrise) were retained in the model if they were significant at $P < 0.10$. ANCOVA was then used to test explicitly for a site difference and to generate least squares means for graphical presentation of metabolite levels controlling for the continuous variables retained by the backward selection procedure. The correlation matrix of metabolite concentrations was used for principal components analysis, and MANOVA of PC1 and PC2 scores was used to test for differences between sites in multivariate space. To investigate possible difference in diet lipid quality (TRIG : PL) between sites we used ANCOVA with TRIG ($\log_{10}+1$) as a dependent variable, site as an independent variable, and PL as a covariate. This avoided the analysis of ratios, and site differences were only considered meaningful if there were no interaction between site and PL, and if PL were a significant covariate. Statistical analyses were performed with SAS 8.02, and metabolite differences between sites in ANCOVA were considered significant at $\alpha = 0.017$, taking into account a Bonferroni correction for six species comparisons and one-tailed predictions about the direction of metabolite differences between sites.

Table 2: Concentrations (mmol/L) of triglycerides (TRIG), glycerol (GLYC), B-OH-butyrate (BUTY), and phospholipids (PL) in plasma of migratory birds sampled at the base and tip of the Long Point peninsula, Ontario, Canada

	BASE				TIP			
Species	TRIG	GLYC	BUTY	PL	TRIG	GLYC	BUTY	PL
HETH	3.35 ± .27	.48 ± .03	.55 ± .05	7.94 ± .27	2.45 ± .18	.46 ± .02	.85 ± .06	8.83 ± .33
WTSP	3.62 ± .44	.64 ± .08	.87 ± .07	8.65 ± .23	2.38 ± .31	.57 ± .04	1.66 ± .15	9.02 ± .22
AMRO	1.89 ± .23	.23 ± .04	.80 ± .11	4.86 ± .53	1.01 ± .12	.41 ± .06	1.21 ± .11	3.95 ± .33
GRCA	1.17 ± .10	.45 ± .04	.89 ± .08	6.90 ± .47	1.16 ± .14	.42 ± .03	.94 ± .08	6.80 ± .65
MAWA	1.72 ± .24	.55 ± .04	1.14 ± .15	7.18 ± .48	1.01 ± .06	.71 ± .08	1.82 ± .38	6.86 ± .59
SWTH	2.14 ± .22	.50 ± .08	.88 ± .14	8.56 ± .93	2.46 ± .22	.63 ± .06	.90 ± .09	9.71 ± .44

Note. Species abbreviations and samples sizes as in Table 1. Species are ordered from top to bottom according to overall median date of capture.

Results

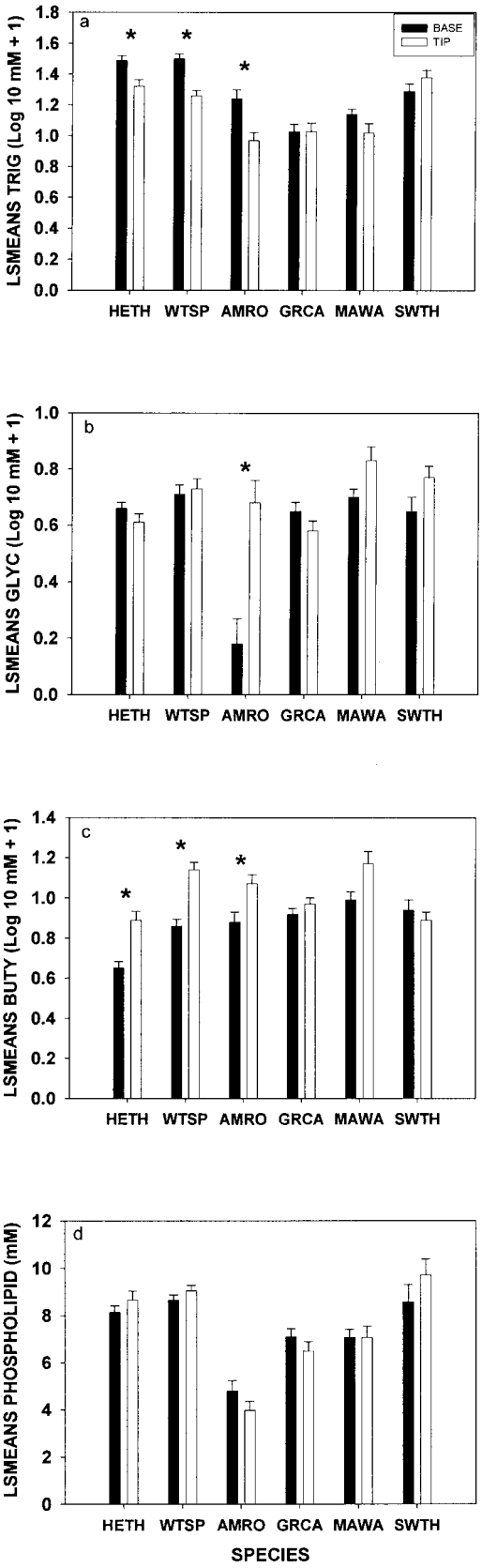
Sample sizes varied from 8–48 at each site, and a period of cold weather in early May delayed the passage of some species (Table 1). Hermit thrushes, white-throated sparrows, and American robins were mainly captured early in the season, whereas gray catbirds, magnolia warblers, and Swainson's thrushes were generally sampled later. Controlling for body structural size, body mass was greater at the BASE in hermit thrushes ($F_{1,68} = 10.08$, $P = 0.002$) and greater at the TIP in Swainson's thrushes ($F_{1,25} = 5.04$, $P = 0.03$). Prior analysis by Dunn (2001) of data collected between 1980 and 1996 indicated superior spring refueling conditions at the BASE for the six bird species except possibly Swainson's thrush (Fig. 2*a*). Regression analysis of our capture data (blood-sampled birds only) supported Dunn's conclusions for some species, but significant gains or losses were difficult to detect with the small sample (e.g., American robins; Fig. 2*b*). Hermit thrushes gained mass at the BASE ($T_{1,42} = 3.0$, $P = 0.005$) and lost mass at the TIP ($T_{1,23} = -2.62$, $P = 0.015$; Fig. 2*b*). Mass gain by magnolia warblers at the BASE was marginally nonsignificant ($T_{1,24} = 2.02$, $P = 0.055$). Site differences in mass change (site by time since sunrise interactions) were detected for hermit thrushes ($F_{1,66} = 12.11$, $P = 0.0009$) and magnolia warblers ($F_{1,32} = 5.82$, $P = 0.022$) and were marginally nonsignificant in white-throated sparrows ($F_{1,85} = 3.77$, $P = 0.056$; Fig. 2*b*). Each case suggested better refueling conditions at the BASE. For two of the late season migrants (gray catbird and Swainson's thrush), there was no evidence of a difference in refueling performance between sites.

Raw plasma metabolite concentrations are presented in Table 2, and the variables retained by multiple regression with backward selection at $P < 0.10$ for each metabolite and bird species are shown in Table 3. Bleed time only affected BUTY measurements. Body mass had a consistent positive effect on TRIG. When time since sunrise was significant, its effect was generally positive for TRIG and PL (except Swainson's thrush PL) and negative for GLYC and BUTY, consistent with a shift in metabolite levels following morning feeding. TRIG concentration was higher at the BASE than at the TIP in hermit thrushes ($F_{1,67} = 7.53$, $P = 0.008$), white-throated sparrows ($F_{1,86} = 22.97$, $P < 0.0001$), and American robins ($F_{1,15} = 11.43$, $P = 0.004$; Fig. 3*a*). Plasma GLYC was lower at the BASE in American robins ($F_{1,14} = 12.18$, $P = 0.008$) and approached significance in magnolia warblers ($F_{1,32} = 5.21$, $P < 0.03$; Fig. 3*b*).

Table 3: Summary of the variables retained at $P < 0.10$ in multiple regression models with backward selection

Species	TRIG	GLYC	BUTY	PL
HETH	+M, +S, +T	+D, −T	+B, −S	+M, +T
WTSP	+M, +S, +T	−T	+B, −M, −S, −T	…
AMRO	+M, +S	−D, −S	−S	…
GRCA	−D, +M	−D	+B	−D, +M
MAWA	+D, +M, +T	−D, +M, −S, −T	+M, −S, −T	+M, +T
SWTH	…	+M, −T	−B, −D, −T	−D, −T

Note. TRIG = triglyceride, GLYC = glycerol, BUTY = B-OH-butyrate, PL = phospholipid, B = bleed time, D = date, M = body mass, S = study site, and T = time since sunrise. Ellipsis dots indicate none of the measured variables explained variation in the plasma metabolite concentration. Plus or minus indicates direction of effect or how sites differed (BASE relative to TIP). Species abbreviations as in Table 1. Species are ordered from top to bottom according to overall median date of capture.



BUTY was lower at the BASE in hermit thrushes ($F_{1,67} = 18.63$, $P < 0.0001$), white-throated sparrows ($F_{1,85} = 26.54$, $P < 0.0001$), and American robins ($F_{1,15} = 7.24$, $P = 0.017$) and approached significance in magnolia warblers ($F_{1,28} = 5.39$, $P < 0.03$; Fig. 3*c*). Plasma PL concentration did not differ between sites in any species (Fig. 3*d*).

Plasma PL was positively correlated with TRIG in all species ($r = 0.39$ to 0.74, $P < 0.001$). There were no significant interactions between site and PL ($0.20 < P < 0.92$) in all species except the magnolia warbler ($P = 0.05$). Least squares mean TRIG controlling for PL was higher at the BASE than at the TIP in hermit thrushes ($F_{1,71} = 18.53$, $P < 0.0001$) and white-throated sparrows ($F_{1,92} = 24.45$, $P < 0.0001$) and nearly so in American robins ($F_{1,15} = 7.0$, $P = 0.018$; Fig. 4).

Principal components analysis was used to combine information from plasma TRIG, BUTY, and PL concentrations. GLYC was omitted from the analysis because it was not correlated with other metabolites in a consistent way among species. In fact, the relationship between GLYC and TRIG was U-shaped (Fig. 5); hermit thrush and white-throated sparrow data are shown because samples sizes were large and the relationships were nearly identical. The first and second principal component axes (PC1 and PC2) explained 88%–93% of the variation in the data. TRIG and PL loaded strongly positive on PC1 in all species, whereas BUTY loaded strongly negative in all species except Swainson's thrush. Thus PC1 represents a mass gain and loss axis. PC2 was difficult to interpret functionally, but BUTY and PL loaded positively on PC2 in all species. In general agreement with the single metabolite results, MANOVA indicated that multivariate metabolite profiles differed significantly between sites in American robins, hermit thrushes, and white-throated sparrows (Fig. 6*a*–6*c*). A pattern suggesting better refueling performance at the BASE was indicated in magnolia warblers, where only birds at the BASE had highly positive PC1 scores (Fig. 6*d*). A larger sample of magnolia warblers at the TIP may have allowed detection of a site difference. There was no suggestion of variation in refueling performance in gray catbirds or Swainson's thrushes (Fig. 6*e*, 6*f*).

Discussion

The results of our analysis validate the use of plasma metabolite profiling under field conditions to assess stopover refueling performance in migrant birds. The difference in site quality at

Figure 3. Plasma metabolite concentrations (+SE) of migratory birds refueling at the BASE and TIP study areas of Long Point. Species abbreviations and sample sizes as in Table 1. Species are ordered from left to right according to overall median date of capture. Values are least squares means of triglyceride (*a*), glycerol (*b*), B-OH butyrate (*c*), and phospholipids, controlling for body mass, date, time of day, and bleed time as needed (*d*; Table 3). An asterisk indicates a significant difference between BASE and TIP.

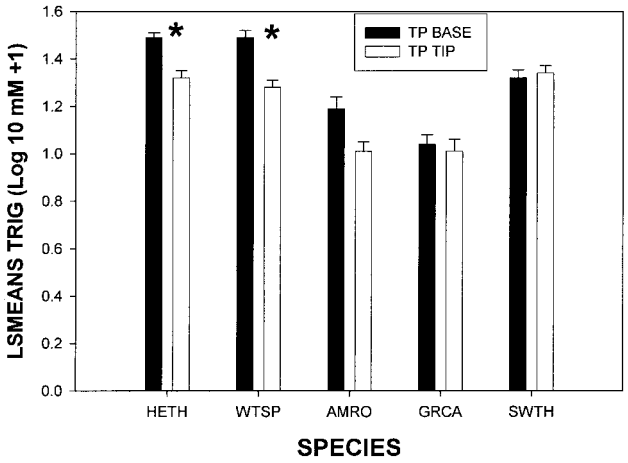


Figure 4. Least squares mean plasma triglyceride (+SE) controlling for plasma phospholipid concentration in migratory birds refueling at two study areas on Long Point. Species abbreviations and sample sizes as in Table 1. Species are ordered from left to right according to overall median date of capture. An asterisk indicates a significant difference between BASE and TIP.

Long Point was thoroughly documented in advance of our study by a regression analysis of 17 yr of capture data (Dunn 2000, 2001). Moreover, regression analysis of our limited sample confirmed site quality differences for some species. Single metabolites showed distinct patterns that agreed with the predicted difference in refueling performance of birds stopping at the BASE and TIP. In particular, TRIG, a good indicator of fat deposition, was higher at the BASE in three early migrating species, while BUTY, a metabolite produced during fat catabolism, was significantly lower at the BASE in the same three species. It is noteworthy that in no case did any of the plasma metabolites differ significantly between sites in a direction that would suggest better refueling conditions at the TIP. Principal components analysis combines metabolite measurements into an overall index or metabolite profile, and this approach further supported the conclusions of our univariate analyses. Most importantly, in most cases metabolite profiles proved to be more sensitive at detecting site differences than regression of size-corrected body mass on time of day. For example, with a small sample of American robins at each site, regression variance was too great to detect a difference between the BASE and TIP, yet plasma metabolite profiles clearly indicated better conditions at the BASE.

Refueling conditions at the TIP appeared to improve later in the spring. Early arriving migrants refueled faster at the BASE than at the TIP, but by late May we could detect no difference in metabolite profiles in species with similar feeding habits to those that passed earlier (e.g., American robin vs. gray catbird and hermit thrush vs. Swainson's thrush). The exposure of the TIP to cold lake conditions is thought to delay the emergence of plants and invertebrates from dormancy in spring (Dunn 2000). Even though magnolia warblers also stopped over at the sites in May, they still performed worse at the TIP, suggesting that feeding conditions for many warblers (foliage insects) may be slower to improve through the season than for the ground-feeding species. As one might expect based on the relative uniformity of physiology among species, metabolite profiling was effective in species with a variety of dietary preferences. To what extent diet nutrient composition, especially fat content, affects the relationships between plasma lipid metabolite levels and realized refueling rate is unknown and should be a focus for future experimental study.

The Information Content of Plasma Phospholipid

In previous work (Guglielmo et al. 2002*b*) we found that plasma PL could reach very high concentrations (higher amounts of fatty acids than plasma TRIG) during stopover refueling in western sandpipers, and we posited that dietary PL may be absorbed and transported in this form. Since the ratio of TRIG to PL varied seasonally, perhaps related to variations in TRIG : PL of invertebrate prey (Hill et al. 1992), we suggested that measuring both TRIG and PL would improve the sensitivity of metabolite profiling and provide information on diet quality. For example, if diet TRIG : PL is low, birds may have to capture more individual prey to achieve a high rate of fatty acid intake. In the present study, plasma PL did not differ significantly between sites, and so it did not appear to contribute much information. However, PL correlated positively with TRIG in all species, indicating that some dietary or endogenously synthesized lipid is transported in this form. Controlling for PL as a covariate, TRIG was higher at the BASE than at the TIP in several species, suggesting that invertebrates

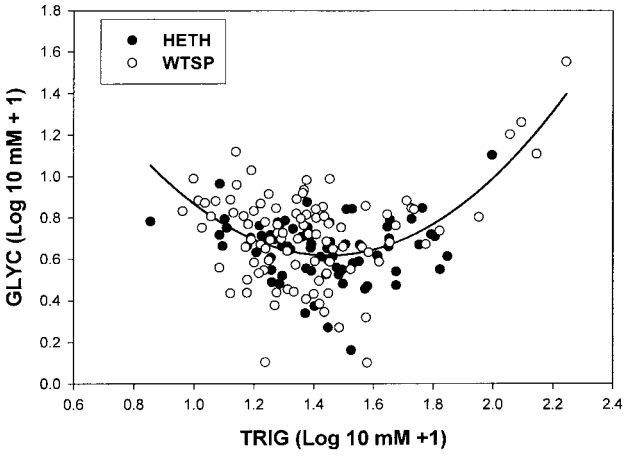


Figure 5. The relationship between plasma glycerol and triglyceride, combining data for hermit thrushes (*HETH*) and white-throated sparrows (*WTSP*). The predictive equation is $y = 3.21 - 3.58X + 1.23X^2$ ($F_{2,166} = 36.18$, $P < 0.0001$).

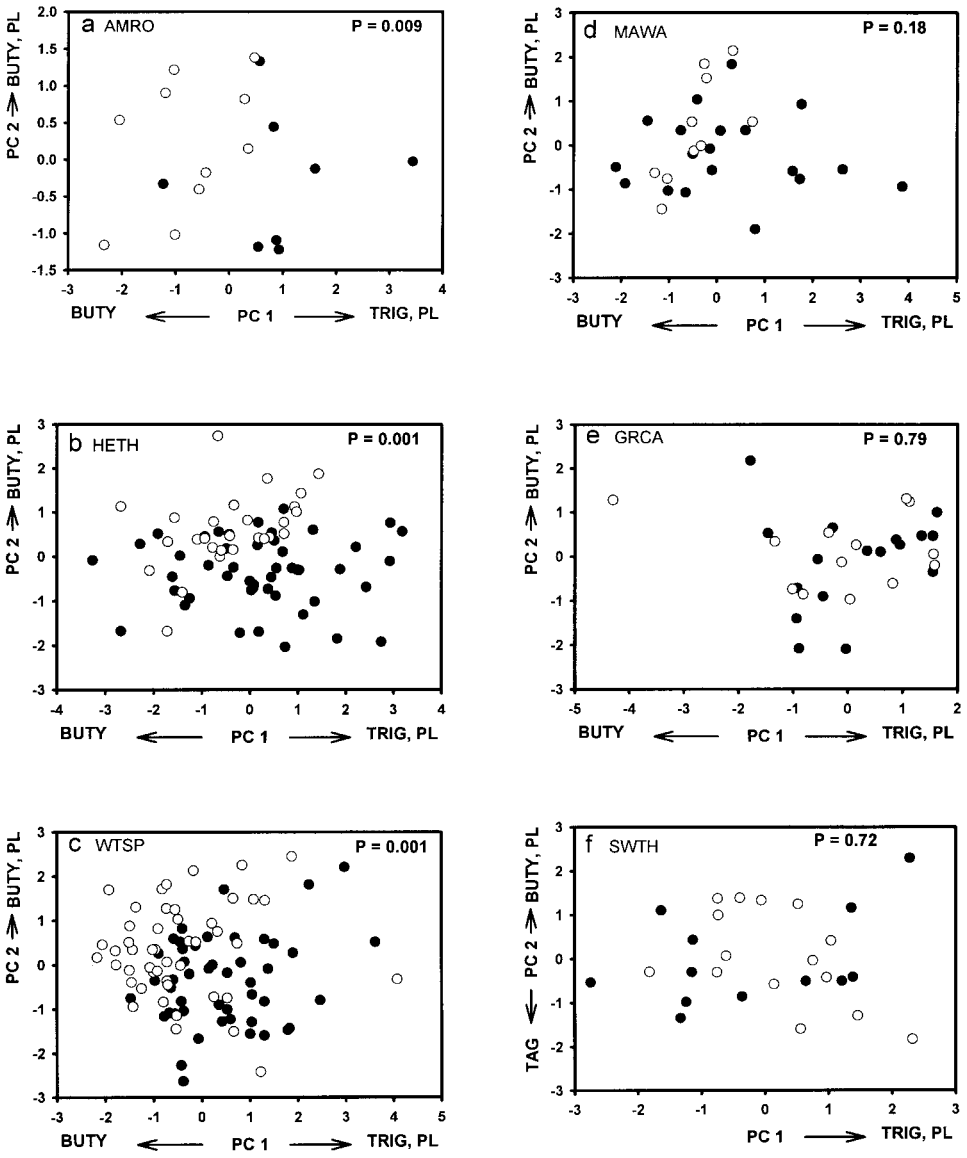


Figure 6. Biplots of PC1 and PC2 scores of plasma metabolites of migratory birds refueling at the BASE and TIP study areas on Long Point. Species abbreviations as in Table 1. Closed and open symbols represent birds sampled at the BASE and TIP study sites on Long Point, respectively. Probabilities are derived from Wilks's λ statistic of MANOVA and test for a difference between BASE and TIP.

may have been of higher quality (higher TRIG : PL) at the BASE. Nevertheless, our analysis may raise more questions than it answers, and controlled studies are needed to determine the relationship between dietary and plasma TRIG : PL. Our prediction that invertebrates have higher TRIG : PL at the BASE than at the TIP early in the spring can also be tested.

A Dual Role for Plasma Glycerol

Glycerol has been considered to be an indicator of fatty acid mobilization because it is released by adipocytes during lipolysis (Jenni-Eiermann and Jenni 1991; Williams et al. 1999; Guglielmo et al. 2002*a*). In captive western sandpipers, plasma GLYC was strongly negatively related to mass gain and was suggested to be an indicator of poor feeding conditions (Williams et al. 1999). In our study, plasma GLYC was negatively related to TRIG at low to moderate TRIG concentrations, but plasma GLYC was positively related to TRIG above ca. 4 mmol/L TRIG. This U-shaped pattern probably reflects increased GLYC production during lipolysis at low TRIG concentrations and during rapid fatty acid uptake by adipose tissue and muscle when plasma TRIG is very high. Adipose (ALPL) and muscle

lipoprotein lipase (MLPL) release GLYC into the plasma as TRIG is hydrolyzed at the endothelial surface, and thus extremely high rates of fat deposition may result in elevated plasma GLYC. There are two major implications of these results for metabolite profiling studies. First, because plasma TRIG assays generally measure total GLYC following lipase hydrolysis, free GLYC must be measured separately and subtracted from total GLYC. Otherwise, plasma TRIG may be substantially overestimated, reducing the sensitivity of metabolite profiling to mass loss situations and exaggerating mass gains. Second, plasma GLYC should probably not be included as a variable in metabolite profile analysis because of its dual role in lipolysis and fat deposition unless all measurements are in a range where the relationship between TRIG and GLYC is linear. At a minimum the relationship between GLYC and TRIG should be examined before further interpretation of GLYC. Uric acid measurements may be similarly confounded because high rates of nitrogenous waste production can result from the deamination of dietary protein (indicating feeding) or body protein (indicating severe fasting) (Jenni-Eiermann and Jenni 1991).

Advantages and Disadvantages of Metabolite Profiling

Metabolite profiling offers an attractive alternative to capture techniques for assessing stopover refueling performance of birds and stopover habitat quality. Metabolites are advantageous because they can be more sensitive than regression techniques and thus may require less capture and sampling effort. This may make it possible to survey more sites or habitats quickly. Metabolites only require a single sample, so that useful data is acquired from each bird captured, and there is no bias associated with recapture. This may facilitate tests of hypotheses relating to age or sex effects. A major advantage of metabolite profiling is that it may be used for species such as shorebirds, which are difficult to recapture and do not have predictable, strictly light-dependent feeding habits (Guglielmo et al. 2002*a*). Although metabolite levels change in relation to feeding patterns, this can usually be controlled statistically or by standardizing capture methods. Metabolite data are no more difficult to analyze than capture data, requiring the same consideration of factors such as body mass, time of day, and date.

There are also some disadvantages to using metabolite profiles. Unlike capture approaches, metabolite studies require the time between capture and blood sampling to be known or estimated because capture stress can affect plasma metabolite and enzyme levels (Jenni-Eiermann and Jenni 1991; Guglielmo et al. 2001; Guglielmo et al. 2002*a*). In practice it is not difficult to measure bleed time, and in our study capture effects were minimal if sampling was done in <10 min. Metabolite studies are complicated by the need to sample blood, process it, and store it securely until analysis. Metabolite assays require some extra time, money, and laboratory infrastructure. The major disadvantage of the metabolite approach is that currently it can only give a qualitative measure of mass change. In our study we can only conclude that the BASE is better for refueling than the TIP, but we cannot determine the actual rate of mass change. Recapture or regression methods provide numerical estimates of mass change, but it remains to be demonstrated that these are accurate. In summary, metabolite analysis offers a powerful means to compare refueling performance among individuals, ages, sexes, and stopover sites. Its greatest utility may be in providing a rapid assessment and ranking of alternative stopover sites or habitat types.

The ability to measure stopover refueling rate is vital not only for the advancement of our fundamental understanding of the ecology and evolution of migrants but also for bird conservation. Migrating birds can only be effectively managed during the stopover phase, and thus techniques to identify high-quality habitats (i.e., those that facilitate rapid fueling) are crucial. Metabolite profiling represents an excellent application of physiology to ecological problems and may lead to a better understanding of landscape level processes in avian migration.

Acknowledgments

We thank Bird Studies Canada and the Long Point Bird Observatory (particularly Matthew Hindle, Jody Allair, Jon McCracken, and Charles Francis) for providing logistical support, housing, and assistance during the field study. We are greatly indebted to Mark Drever, Antony Wood, Thomas Nudds, and Paul Hebert of the University of Guelph for laboratory support in Ontario. We are grateful to Erica Dunn for thoughtful advice during the planning stages. We thank two undergraduate students, Samantha Pasternak and Anna Krokfors, for conducting TLC. Funding was provided by the University of Montana Research Grant Program and the U.S. National Science Foundation (REU supplement to IBN-0224954).

Literature Cited

Alerstam T. and A. Hedenström. 1998. The development of bird migration theory. J Avian Biol 29:343–369.

Alerstam T. and Å. Lindström. 1990. Optimal bird migration: the relative importance of time, energy and safety. Pp. 331–351 in E. Gwinner, ed. Bird Migration: Physiology and Ecophysiology. Springer. New York.

Biebach, H., W. Friedrich, and G. Heine. 1986. Interaction of body mass, fat, foraging and stopover period in trans-Sahara migrating passerine birds. Oecologia 69:370–379.

Dunn, E.H. 2000. Temporal and spatial patterns in daily mass gain of magnolia warblers during migratory stopover. Auk 117:12–21.

———. 2001. Mass change during migration stopover: a com-

parison of species, groups and sites. J Field Ornithol 72:419–432.

———. 2002. A cross-Canada comparison of mass change in birds during migration stopover. Wilson Bull 114:368–379.

Guglielmo, C.G., P.D. O'Hara, and T.D. Williams. 2002*a*. Extrinsic and intrinsic sources of variation in plasma lipid metabolites of free living western sandpipers. Auk 119:437–445.

Guglielmo, C.G., T. Piersma, and T.D. Williams. 2001. A sport-physiological perspective on bird migration: evidence for flight-induced muscle damage. J Exp Biol 204:2683–2690.

Guglielmo, C.G., T.D. Williams, G. Zwingelstein, G. Brichon, and J.-M. Weber. 2002*b*. Plasma and muscle phospholipids are involved in the metabolic response to long-distance migration in a shorebird. J Comp Physiol B 172:409–417.

Hentschel, B.T. 1998. Spectrofluorometric quantification of neutral and polar lipids suggests a food-related recruitment bottleneck of juveniles of a deposit-feeding polychaete population. Limnol Oceanogr 43:543–549.

Hill, C., M.A. Quigley, J.F. Cavaletto, and W. Gordon. 1992. Seasonal changes in lipid content and composition of benthic amphipods *Monoporeia affinis* and *Pontoporeia femorata*. Limnol Oceanogr 37:1280–1289.

Jenni L. and S. Jenni-Eiermann. 1996. Metabolic responses to diurnal feeding patterns during the postbreeding, moulting and migratory periods in passerine birds. Funct Ecol 10:73–80.

Jenni L. and R. Schwilch. 2001. Plasma metabolite levels indicate change in body mass in reed warblers *Acrocephalus scirpaceus*. Avian Sci 1:55–65.

Jenni-Eiermann S. and L. Jenni. 1991. Metabolic responses to flight and fasting in night-migrating passerines. J Comp Physiol B 161:465–474.

———. 1992. High plasma triglyceride levels in small birds during migratory flight: a new pathway for fuel supply during endurance locomotion at very high mass-specific metabolic rates? Physiol Zool 65:112–123.

———. 1994. Plasma metabolite levels predict individual body-mass changes in a small long-distance migrant, the garden warbler. Auk 112:888–899.

Lindström Å. and T. Piersma. 1993. Mass changes in migrating birds: the evidence for fat and protein storage re-examined. Ibis 135:70–78.

Moore F.R. and P. Kerlinger. 1987. Stopover and fat deposition by North American wood warblers (Parulinae) following spring migration over the Gulf of Mexico. Oecologia 74:47–54.

Pyle, P., S.N.G. Howell, R.P. Yunick, and D.F. DeSante. 1987. Identification Guide to North American Passerines. Slate Creek, Bolinas, CA.

Rising J.D. and K.M. Somers. 1989. The measurement of overall body size in birds. Auk 106:666–674.

Schaub M. and L. Jenni. 2001. Variation of fuelling rates among sites, day and individuals in migrating passerine birds. Funct Ecol 15:584–594.

Williams T.D., C.G. Guglielmo, O. Egeler, and C.J. Martyniuk. 1999. Plasma lipid metabolites provide information on mass change over several days in captive western sandpipers. Auk 116:994–1000.

Ydenberg, R.C., R.W. Butler, D.B. Lank, C.G. Guglielmo, M. Lemon, and N. Wolf. 2002. Trade-offs, condition dependence, and stopover site selection by migrating sandpipers. J Avian Biol 33:1–9.

Yong, W., D.M. Finch, F.R. Moore, and J.F. Kelly. 1998. Stopover ecology and habitat use of migratory Wilson's warblers. Auk 115:829–842.